In the last fifteen years much progress has been made on the knowledge of the mechanisms controlling the genetic and molecular activities of the mammalian (with special emphasis on the human) heart in chronic cardiac insufficiency.

This increased knowledge has greatly influenced the understanding and treatment of heart failure, helping to prolong duration of life; even if clear evidence of a long lasting improvement of the syndrome is lacking.

The causes of heart failure are numerous and different, but the common feature depends on the modest regenerative capacity of the human heart after any important injury damaging the myocardium. Therefore, the more frequent consequence is the appearance of scar tissue, with collagen deposition and tissue remodelling. Even if some proliferation of cardiac myocytes has been noted, this process is unable to overcome the destruction of normal cells observed in the several cardiac disorders, so that the only efficient response is usually the cellular hypertrophy.

In contrast to mammals, other classes of vertebrate species display an efficient regenerative capacity. Regeneration is a very complex biological process, requiring cellular proliferation, de-differentiation and trans-differentiation, and a well regulated orchestration of the activatory regulation of the stem cells that take place in different ways, according to the tissues and organs where the regeneration is present and specific to the different species of the involved animals. Many authors have described the ability of some vertebrates (the zebra fish is the most extensively studied subject) to regenerate large parts of the heart after an extensive injury (review by SLEEP et al.).

In mammals the myocardial hypertrophy is in some way opposed to the regenerative capacity, with very different long-term consequences. Heart failure develops weeks, months or years after the appearance of the hypertrophy; which is induced by the molecular and the genetic new program in the adult cardiac myocytes and tissues, leading usually to a fatal evolution, with specific mechanisms producing the different types of the cardiac hypertrophy.

The gene expression of the initiation and the progress of cardiac insufficiency have been only partially identified, depending on both the cells signalling in consequences of the initial injury and the growth factors involved.

The research on the genome-based expression have revealed large differences between the systolic (volume overload) and the diastolic (pressure overload) models of hypertrophy, their deregulatory consequences, the different pathophysiological and clinical consequences of the two types of hypertrophy affecting simultaneously. However, not with the same intensity, the cardiac cells, the collagen and the mechanism of contraction and relaxation.

The increase of the load induces specific changes in the expression of many regulatory molecules; contractile proteins, components of the extracellular matrix and ion channels are all able to modify the systolic and the diastolic regulation of the cardiac activity, mainly the contractility (inotropic state), relaxation (lusitropic properties) and their affect on circulation. These changes, initially adaptive and therefore useful, become deleterious in the long time because they are excessive and persistent, and so are viewed as the initial mechanism leading to cardiac insufficiency.

The initial derangements leading to heart failure stem from the need to add new material to, or substitute it to increase the heart mass; in the presence of chronic pressure, volume overload, acutely damaged cells, cells destroyed by circulatory, infarct and related conditions, inflammatory (myocarditis) causes or, when myofibres are not sufficient to meet the needs of circulation (as in valvular or congenital defects) or when the myocytes are chronically replaced, as in an infiltrative process of the muscle wall (amyloidosis, fibroelastosis, etc).
These conditions, through the augmented load on the non-affected tissues, induce the cellular elongation or stretching (22), which is the initial stimulus to a new genetic program. The genes so affected induce the increase of cellular size (not the number), modify the cytokton and the extracellular attachments, at variance with what happens in normal cellular mitosis (23). The replication of the necessary products is made possible initially by the activation of simple, foetal-type of heavy chains myosin, which shows a slow capacity of contraction and relaxation (24). These phenotypes affect more or less profoundly the velocity of the formation and dissociation of the bridges between the long chain heads of myosin and actin (25). The process results, on the one hand, in the slowing of contractility and, on the other, in the reduction of ATP demolition, the fuel of cardiac action.

The extracellular stimuli that increase fibres elongation (extracellular first messenger), and originate the new genetic program, even if not completely understood, can be described as follows:

- when the stimuli act during systole, the additional sarcomeric proteins are assembled in parallel (26), producing thickening of the ventricular wall and concentric hypertrophy, with relative maintenance of the chamber volume; the replication in parallel impairs mainly the filling of the ventricle mainly through the reduction of wall compliance, while the systolic function is preserved;

- when the stimuli act during diastole the sarcomeres assemble in series (27), dilating the ventricular cavity without increasing individual myocyte length and therefore not distending the single cardiac fibres. An early myocardial dysfunction is produced due to the slow-acting myosin, which reduces the active tension produced by the ventricle and impairs their filling and relaxation, producing a combination of hypertrophy and dilatation.

The difference in the phenotypes of the different type of cardiac hypertrophies suggests that the genes activated by chronic volume or pressure overload are not the same (28), in relation to the different model of the initial stimulus. In both cases, the overload elongating the cardiac fibres, either in systole or in diastole, induces a different re-expression of genes of a foetal/neonatal type.

As the cardiac myocytes are the target of peptide-derived growth factors, the integration of different endocrine, paracrine and autocrine factors during systole and diastole suggest a role of different initial external stimulating factors. Among them, angiotensin II and the cytokines (29) probably produce the response (concentric hypertrophy) to the systolic overload, while insulin-like growth factors and endothelin (30) are probably the mediators of the diastolic overload response—wall hypertrophy and cavity dilatation.

The consequences of the genetic program reduces, through the proliferation of new contractile proteins, the chronically augmented burden imposed by the excess load and the resulting failure of the sarcomeres tension and length. However, the slow-acting myosin so constructed, more or less rapidly, reduces the contractility of the cardiac fibres, interfering with the active tension developed by the heart in toto (31). Thus the hypertrophic heart has a less than optimal capacity for sustaining and modulating peripheral needs, during effort in particular.

Initially, the consequences are matched by the increase of the contracting mass (the initial hyperfunction phase of Meerson), then, after a period of stabilisation (stable hyperfunction), the chronic large pressure load eventually exceeds the capacity of the ventricle, and the heart dilates (exhaustive phase of Meerson), in clinical terms presenting as the appearance of the insufficiency of the cardiac pump (32).

The complex mechanism of genetic activation is produced by the re-entry of the adult cardiac myocytes, which have lost the capacity for replication, into the cell cycle, through the over expression of oncogenic substances (33), associated with the down-regulation of other usual promoters. (Table 1) (34) Re-expression and up-regulation are associated with intense activity of some coupling receptors, such as Gq-coupled receptors, calcium receptors, some protein kinases, and a adrenoceptor. (35) In this way the mitogen-activated cascade promotes a very early and important proliferation of the proteins of the myocytes and of the collagen.

Table 1: From the literature, phenotypic changes of different components of human myocyte in heart failure

<table>
<thead>
<tr>
<th>Increase (↑), decrease (↓), no change (=), no data available (0), controversial (?)</th>
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<tr>
<td><strong>Membrane receptors</strong></td>
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<td>Rianodine receptor</td>
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<td>Myofilibrar ATPase</td>
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<td><strong>Membrane receptors</strong></td>
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<td>β1-AR</td>
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<td>Adenylate cyclase</td>
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<td>Ca²⁺ ATPase of SR</td>
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<td><strong>Sarcomere proteins</strong></td>
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<td>Fetal MLC reexpressed</td>
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<td><strong>Energetics</strong></td>
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<td><strong>Energetics</strong></td>
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<td>Isocreatinine Kinase shift</td>
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<td><strong>Sarcomere proteins</strong></td>
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<td>M2-R</td>
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<td>Adenosine R</td>
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<td>Shift to α3-subunit</td>
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These factors induce, in turn, the stimulation of the second intra-cellular messenger, whose pathways include G protein-coupled receptor and the receptor for tyrosine kinase. The ras-dependent proto-oncogen factor is probably the most important mediator, selectively up-regulating some substances as myosin light chain 2, and re-expressing others as atrial natriuretic factor (ANF). The gradual substitution of normal contractile proteins by the foetal/neonatal type is the cause of a shorter lifespan of most myofibres, so inducing an accelerated cellular death, through apoptosis and necrosis.

The profound changes in the contractile apparatus, that reduce the contractility, further limiting the possibilities of the reversible heart failure. The total production of energy is probably depressed, even though conflicting data have been reported, at least in the initial phases of heart failure. When the mitochondria of failing hearts are studied, a reduction of the cytochrome content and activity is found, principally because of an inadequate matching of the mitochondria with the needs of the contractile apparatus. Moreover, creatine-kinase activity and the creatine and phosphocreatine content of the failing heart are depressed. In conclusion, myocardial energy and its reserves are reduced, further limiting the possibilities of the heart in the initial stages of failure.

The stretched myocardial cells express the receptor angiotensin II, which binds to several different effectors, with the intervention of the regulatory protein G 1 which in turn regulates the movements of the ion Ca inside the cardiac cells. Calcium and C protein kinase activate the genes responsible for the development of augmented shape of the myocytes, usually expressed only in the foetal heart.

The activated genetic expression is so depending upon many diversified activities:

a) genes with immediate expression as ras, c-fos, c-jun and fetal genes regulating the myosin isoforms, as ANF, alfa skeletric actin, heavy chains myosin alfa and beta Gq, gp 130, able to induce the formation of heavy myosin;

b) genes controlling the cytoskeleton, as the extracellular matrix, as fibronectin, collagen II, binded to metalloproteases (MMP) which promote and increase the development of the supporting system of the heart;

c) little increase of the mitochondrial system, with a small increase of NADH, linked to the augmented activity of the high energy phosphate system and augmentation of intramitochondrial Ca;

d) Genes encoding the ion channels, contributing to the electrical instability of the ventricles.

The resulting new phenotype is produced by the uneven activity of the several energetic systems making excess production of the contractile and modulating proteins in relation to the energetic system and to the development of the vessels. Moreover, according to the type overload (systolic or diastolic) the increase in the shape of the cardiac cells is the result of the replication of the myofibris in parallel or along the major axis of the cells (apposition in series).

The intervention of the new genetic program of the adult myocardial cells has profound implications on the anatomical and physiological activities of all the intracellular components if the myocardial cells, modifying both the proteins regulating the contractility and the anchoring properties. The general mechanism is the sum of the numerous signal-transduction cascade protein coupled receptor signalling pathways (the so called stimulus-response coupling), among them the G proteins play the most prominent role.

**Figure 1** shows the interaction of the respective receptors with G protein heterotrimer.

The changes of the several classes of myocytic proteins help to explain the progress from the simple cellular dysfunction to the irreversible heart failure.

The contractile proteins, actin and myosin, are both deeply modified by the resumption of genetic program by the injured adult hearts, reducing the velocity of formation and dissociation of the cellular cross bridges, leading to a more or less important reduction of the inotropism.

The changes of the modulating proteins are less marked, even if the coupling is slowed by the reduced affinity of troponins, tropomyosin and with the Ca ions. More important are the changes of the large anchoring proteins, titin and A Kinase anchoring protein (AKAP), which moreover becomes not uniformly distributed inside the cells. As they trigger the myocyte to contract, their change reduces further the inotropism.

The pool of the CAMP-dependent PKA relies upon the selective stimulation of the adrenergic system. The opposite effect the adenyl cyclase and phosphodiesterase generate non uniform a-AMP and produce compartmentalised a-AMP: Some molecules interact with the PKA holoenzymes, the so-called AKAP, which serve as regulatory enzymes for the cAMP machinery, participating to the sympathetic and parasympathetic regulation of cardiac contractility.
of calcium into the sarcoplasmatic reticulum.

menting the phosphorilation of phospholamban and the reuptake of calcium into the SR. The AKAP15/18δ long isoform brings PKA into complex with phospholamban and SERCA2, where it can augment the phosphorylation or phospholamban and the reuptake of calcium into the SR.

This mechanism helps to open the L type Ca channels, due to local increase of the calcium ions; followed by the active transport of the ion back into the lumen of the sarcoplasmatic reticulum, initiating the myocyte relaxation.

No less important are the changes of the cytoskeleton, the supporting apparatus of the myofibres, characterised by the increase and different composition of the collagen, producing a precocious fibrosis which modify the metabolic exchanges through the tissues surrounding the capillaries, reducing their angiogenesis and the perfusion of the hypertrophied cardiac cells. In addition, in both the systolic and diastolic overloads, fibrillar types of collagen I and III mRNA increase.

In addition the matrix metalloproteases (MMPs), important in the reasorption of the extracellular matrices, are activated in presence of cardiac dilatation, and simultaneously their tissue inhibitor (TIMP) is down regulated, contributing to the negative remodelling of the heart in cardiac insufficiency.

At the end the normal cardiac cells undergo a process of polyploidi- sation instead of the conventional mitosis. The reduced duration of the life of these cardiac cells accelerates the substitution of the normal with the abnormal myocytes.

In brief, the interplay between the new molecular arrangement and the new genetic activity is the main mechanism producing the pathophysiological changes of the heart failure, with its progressive course.

Moreover, the occupancy of the beta receptors stimulate the cAMP signalling pathway to activate protein kinesis, guanine nucleo- tidies and ion channels. These substances compartmentalise the responsive enzymes Ca and AMP, mainly in the pacemaker cells, participating in the sympathetic and parasympathetic regulation of cardiac contractility. These proteins, named AKAP, help to position the cAMP responsive enzymes in proximity of their substrates, augmenting the phosphorilation of phospholamban and the reuptake of calcium into the sarcoplasmatic reticulum.

The changes of the electromechanical coupling, is the initial mechanism governing the abnormalities of the contraction and the relaxation of the myocites. Therefore, the adverse genetic and molecular profiles are the unfavourable factors regulating the myocites environment as the most important contributor to the origin and development of the heart failure.

The progress of the knowledge on the genetic and molecular changes induced by the correction of the mammalian cardiac injury has proved able to identify the mechanisms governing the evolution of the heart toward the initial dysfunction, leading to the development of cardiac insufficiency, through the reduction of the inotropic power, the impaired relaxation, the increase afterload and its mismatch with preload and contractility.

The increase of knowledge in the relation between the bench research and the bed applications have made possible the development of new more powerful techniques, pharmacological, electrical, mechanical and surgical aiming to improve the doomed course of the cardiac insufficiency.

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