

Electric Impedance Recording - A Noninvasive Method of Rejection Diagnosis

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Abstract

The recording of electric tissue impedance reflects membrane function and changes of volume and electrolytes in intra- and extra-cellular space. The purpose of this study was to determine if acute cardiac allograft rejection can be diagnosed from alteration of electric transmyocardial impedance (ETMI). Nine Beagle dogs received a heterotopic neck heart transplant. The animals were immunosuppressed with cyclosporine and steroids for the first three postoperative days. ETMI was recorded twice daily. Transcutaneous biopsies were performed whenever the impedance varied by more than 10%. All hearts showed a uniform impedance decrease immediately after transplantation, reaching a stable plateau after three days. A subsequent increase of impedance by $12.4 \pm 2.9\%$ was accompanied by a histological diagnosis of mild rejection. A further increase of $23.2 \pm 2.6\%$ histologically showed acute moderate rejection. After treatment with steroids the impedance reached the basic plateau again. Compared with the biopsy the sensitivity and specificity were 100%. The results indicate that the inflammatory process of cardiac allograft rejection can be detected with this method. This technique will be clinically applicable when an implantable telemetric device for recording the impedance and its transmission will be developed.

Introduction

To investigate the integrity of cell function in biologic tissue, there are varying methods:

1. Biochemical analysis of reaction products of cell metabolism
2. Investigation of the potential course of cellular membrane during depolarization and repolarization, and
3. Measuring bioelectric impedance or its inverse value - conductivity.

As bioelectric impedance is easily registered with modern electronics and therefore can be applied to man noninvasively, this method is being further developed for the determination of organ rejection. Ideas about the way in which biologic impedance can be determined in a physical

model have been developed by various authors already during the last century, using the Potential-Theory of dielectric for biologic tissue [1,2]. For this purpose the biologic tissue is considered as a cell suspension within an electrolyte fluid, which represents the extracellular space (Fig. 1). The single cells with their membranes are considered as having the quality of a dielectric in an electric conductor as it is used as insulator in condensers [3]. Models by various authors differ mainly in the hypotheses on geometry of the cell. Some consider cells spheric formations, while others assume that they have cylindric or oval shapes [4,5,6]. Mathematical evaluation of these models allows a rough estimate of impedance values and lead to the recognition that 1. the cell membrane mainly supplies capacitance to the total impedance of a cellular suspension 2. the dielectric constant of biological tissue is dependent on frequency and 3. the value of the measured impedance is a function of frequency and wave-form of the applied current. Another dependent factor is the shape and surface of the electrode used for measuring, as could be shown in practical experiments [7,8]. The inflammatory process caused by organ allograft rejection after transplantation leads gradually to lysis of organ-specific cell structures. The various grades are determined by the examination of lymphocyte infiltration and electrolyte changes among intra- and extra-cellular space. In order to examine the suitability of impedance measurement as an indicator of rejection, we measured ETMI on heterotopic transplanted neck hearts of dogs.

Methods

Nine beagle dogs (16 kg bw) underwent heterotopic neck heart transplantation. The transplanted heart was equipped with four screw-in electrodes, which were led subcutaneously to the surface. Immunosuppression consisted of 250 mg methylprednisolone on 3 consecutive days and one intraoperative dose of 250 mg. Preoperatively, 10 mg/kg bw cyclosporine was given and then basic immunosuppression was adjusted to 400 ng/ml whole blood level (nonspecific measurement). Twice daily, impedance was measured according to the two pole method. Biopsies were taken after impedance values had reached a stable plateau, and afterwards whenever impedance values had changed by more than 10%. The stable impedance plateau was, at the same time, the starting point for reduction of

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cyclosporine basic immunosuppression on whole blood levels of 100 ng/ml to induce allograft rejection. Seven dogs were killed after impedance increased, which corresponded to biopsy findings of grade 3 rejection according to the classification of the International Society for Heart Transplantation (ISHT) [9]. Two dogs were treated for rejection at this point by additional immunosuppression of 250 mg methylprednisolone on 3 consecutive days and an increase of cyclosporine level to values of 400 ng/ml. Biopsy frequency was further dependent on impedance values as described above.

Technique

With support of modern electronics, there are manifold possibilities to measure impedance. The classic (4 pole) method applies a sinusoidal current over a bipolar electrode into the myocardium [10]. Magnitude and phase of the appropriate voltage are measured with an additional bipolar electrode. This procedure has the advantage that there is hardly any current over both electrodes that measure voltage, so that polarization effects on electrodes are hardly relevant, and absolute values of impedance can be determined exactly.

As our investigations focused on monitoring the course of daily impedance values, we used the two pole method. Rectangle impulses of various impulse widths (1 ms to 10 ms) are applied over a bipolar electrode, and over the same electrodes the impulse response is registered. The day-to-day comparisons are based on analysis of maximal amplitudes of impulse responses of similar impulse widths (Fig. 2). Polarization effects that are more intensive when using this method lose their significance because only relative impedance values of the day-to-day examination have been considered. By calculating the differences, the constant daily values that are caused by polarization are excluded from the evaluation. Two examples may further describe the impulse method. A rectangular impulse is transmitted to a resistance circuit (Fig.3). At the output of this circuit the impulse must be measured in the same form but in a lesser amplitude. A circuit consisting of resistances and a condenser (Fig. 4), which could also be considered as a model of a myocardial compartment, modifies the rectangular impulse in form and in amplitude. Changes in form are dependent on resistance values and the capacitance of the condenser. This means: by determining the impulse response curve, one obtains information on capacitive and ohmic qualities of the circuit.

Results

With the mode of presentation explained above (Fig. 2) the curve representing the results of relative impedance for dogs which were killed after grade 3 (ISHT) rejection is obtained (Fig. 5). During the first 3 days after heterotopic transplantation one can see a marked decrease of relative impedance, until a stable plateau is reached. A biopsy

taken at this moment does not show signs of rejection. A further biopsy taken after an impedance increase of more than 10% was classified as Grade-1 (ISHT) rejection, and a biopsy after impedance increase of more than 22% as Grade-3 (ISHT). Table 1 represents mean values of impedance measured in seven dogs.

In both dogs which received treatment for Grade-3 rejection with steroids, a new decrease of impedance of 11% occurred (Fig. 6). A biopsy taken simultaneously at this impedance level showed clear signs of recovery, however with still active lymphocytic infiltrate requiring further treatment. After further treatment with steroids impedance was decreased to the initial level. At this time, bioptic findings showed complete recovery of the myocardium without lymphocytic infiltration.

Table 1
Relative impedance increase: average of 9 animal experiments

Biopsy	Relative impedance increase
Mild rejection (ISHT grade 1)	12.2% $\pm$ 2.3%
Moderate rejection (ISHT grade 3)	20.8% $\pm$ 3.1%

Discussion

Although animal experiments of impedance investigations were already done since the 1920s, mainly in the field of impedance cardiography, and in the 1950s also with the aim of obtaining hemodynamic parameters, no impedance measuring method could be clinically established on an extended basis for diagnostic purposes [11]. One reason may be the required great expense in technology with a high degree of precision, to an extent that became only possible with the development of electronics in the last 20 years. Another reason may be seen in the fact that very low impedance changes are to be detected, which — and this increases the difficulty — are subject to physiological variations.

Technology

Up to now intracorporeal impedance has been measured only experimentally. Compared with impedance measurements of other organs, measuring transmyocardial impedance presents the special problem that the measuring current must not excite the heart, as this could lead to fibrillation or to asystoles. The current must therefore not be allowed to exceed 10 micro amperes. When intending to measure impedance values of magnitude and phase with a sinusoidal current at the myocardium, care must be taken

of the excitation or depolarization phase of the physiologic excitatory process. This means that the time which is available for slow sinusoidal wave can set a lower limit to the frequency of applied current. It is, however, known that impedance with measurements of low frequencies is mainly of interest when alterations of extracellular space are intended. With regard to this, the impulse method chosen here does not present any problems. The range of impulse widths is within milliseconds, and is therefore in the zero-phase of the ECG easily placed between the t-wave and the p-wave. Apart from this, the rectangular impulse consists of a spectrum of frequencies so that it depends on the chosen moment of the impulse response whether alterations with high or low frequencies can be observed. It is expected that the effect of impedance on high frequencies will be observed predominantly at the starting phase of the impulse response, whereas the effect of impedance on low frequencies will be observed chiefly in the end phase.

Experiment

Histologic signs of rejection after cardiac transplantation are not distributed homogeneously in the myocardium, but appear as focal infiltrates of mononuclear cells [12,13]. A false negative biopsy diagnosis is possible due to sampling error [14]. Assuming that impedance measures the same changes as determined by histomorphologic examination, i.e. lymphocytic infiltrate and edema, it is important to place the electrodes in such a way as to leave sufficient space between them. Intraoperative impedance measurements in cardioplegic hearts are performed by Preusse [15] to examine ischemic stress with impedance measurements. His results showed an impedance increase during extended ischemia. During rejection after cardiac transplantation effects similar to ischemia occur when the rejection process is concurrent with distinct vasculitis [16,17,18]. However, it cannot yet be determined whether all forms of rejection processes are accompanied by vasculitis, which, for example, may be the cause for low voltage of intramyocardial electrograms in cases of rejection [19]. It is probable, however, that the initial decrease of impedance is due to ischemia during transplantation and to reperfusion damage. Whether impedance increases during rejection that is initiated through reduction of immunosuppression, "only" caused by ischemia or also by cellular damage, edema, and cellular infiltration, cannot yet be shown. However, it can be observed that during the early stage of rejection and also during the treatment phase, biopsy classifications can be applied to impedance measurements.

Conclusions

These investigations in animals showed how far inflammatory processes of rejection are reflected by changes in myocardial impedance. Further examinations must confirm long-term stability of measurements. Electrodes present a special problem. Screw-in electrodes can give

more stable measurements, but have the disadvantage of creating more scarring than other electrodes. Other electrodes, such as activated carbon, should be tried to use electrodes with low polarization. Statistic evaluation of these animal experiments concerning sensitivity and specificity could be made, but should not be overestimated regarding consistency. With the aid of an implantable impedance measuring unit which is now being developed it will be possible to obtain impedance values telemetrically, and thus to improve diagnostic monitoring of rejection while the patient is at home. The results found here should be paralleled in clinical research projects with a larger number of patients. The question which cellular alterations in the myocardium cause the changes in impedance remains open. This problem could possibly be further analysed by investigations using wider rectangular impulses.

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Questions and Comments

- Q. You have very nicely shown that there is a good correlation in your experimental study. The question, however, is whether the 12 percent increase in your impedance that detects no to mild rejection. The point we want to get to is to clinically detect the onset of mild rejection because mild will either resolve or go on. That is why we have to know when mild rejection is present, whether that is significant or whether that is in the range of one animal to another. Could you comment on the significance of this change and whether you are confident that you would be able to detect mild rejection just solely by this method?
- A. I think the advantage of this method is to detect mild and moderate rejections in contrast to other electrophysiological methods which can only detect moderate rejections; the significance of this method is difficult to assess due to the amount of the animal experiments. We did only eight experiments and that is not a sufficient number to have good significance.
- Q. Regarding the amount of current used in the impedance. What typically do you use as current running between the two electrodes?
- A. We have to take care that the amount of current does not have an effect on the heart. That means we have to be careful that we don't induce fibrillation or something like that. Range of microampers is 10 to 20.
- Q. The electrodes you used were screw-in electrodes?
- A. We used screw-in electrodes knowing that the polarization effect of these electrodes is not negligible. In the next experiment we will use electrodes

that have a reduced polarization effect.

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- Q. I need a little better definition of what you are measuring. Are these wave-forms being recorded from unipolar or bipolar electrodes?
- A. Between two unipolar electrodes.
- Q. Are you looking at the entire wave-form being recorded from that bipolar electrode or are you factoring out portions of that wave-form?
- A. We factorize the entire impulse response and compare identical components from different impulse responses to each other.
- Q. How can you separate the effects of anisotropy from the waveforms you are recording from the bipolar electrodes, anisotropy being the inhomogeneity of myocardial fiber orientation and direction? And how that inhomogeneity affects wavefront propagation through myocardium? I don't think that you can factor that out and if there is any change in conduction your waveform is going to change by definition. It is a very difficult thing to factor out and I am just wondering how you addressed that. You can factor out resistivity or the ratio of intracellular, extracellular longevity resistance by what you are measuring and you can factor out resting transmembrane potential and that might affect the wave-form, but I am curious to know how you are factoring out the anisotropy.
- A. Well, from my experiments I cannot answer the question, whether anisotropy of myocardial fiber orientation influences the impulse wave-form. But, don't forget, I am only interested in the day-to-day changes of the impedance. The screw-in electrodes are in unchanged position during all measurements, and the fiber orientation between the electrodes will not change. The measurements are also ECG triggered, and the reason for changes conductivity may be changes in impedance as a result of a rejection process.

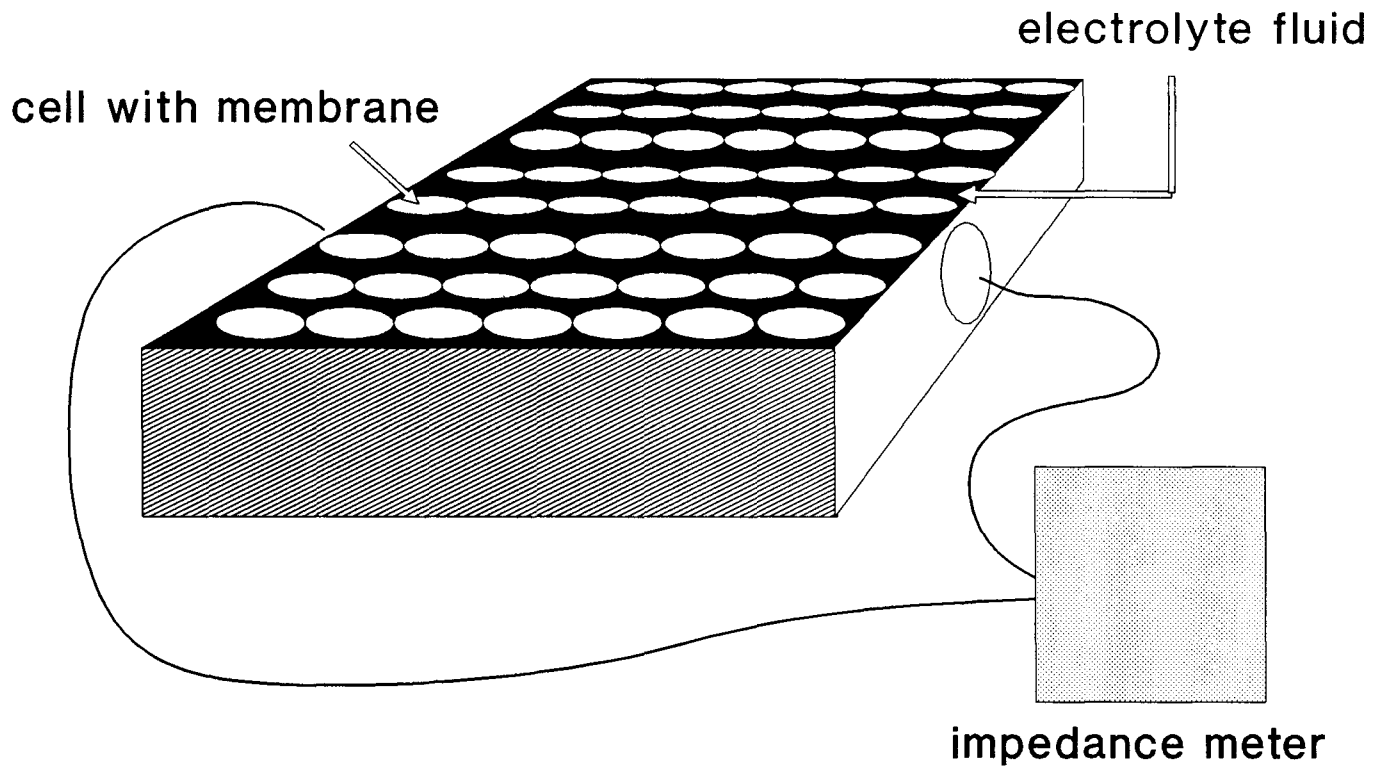


Fig. 1: Schematic presentation of a possible impedance model. The membranes are responsible for a dielectric property of cell structure

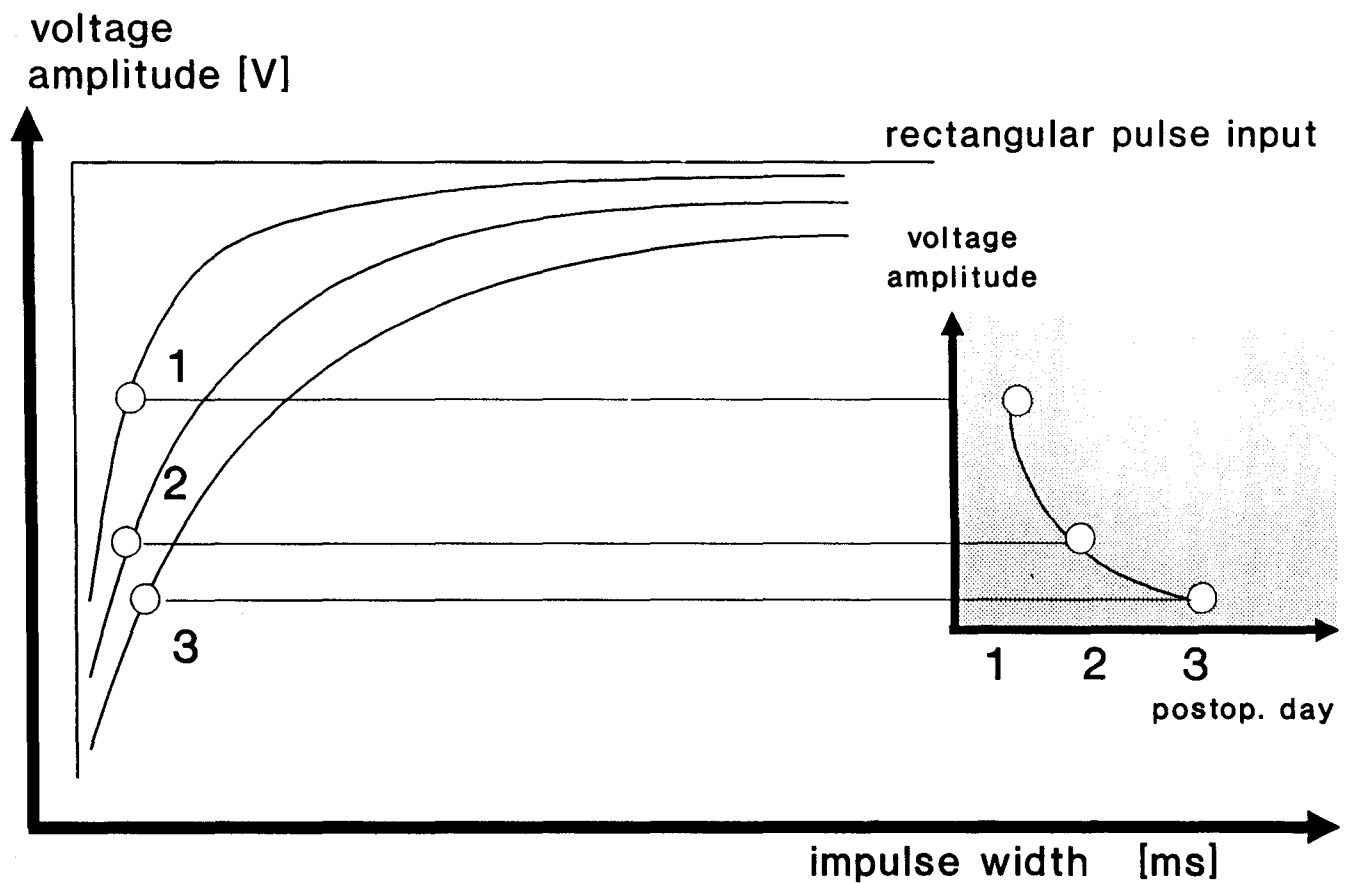


Fig. 2: Impulse response curves at different moments. How to display the impedance curves for fixed impulse width

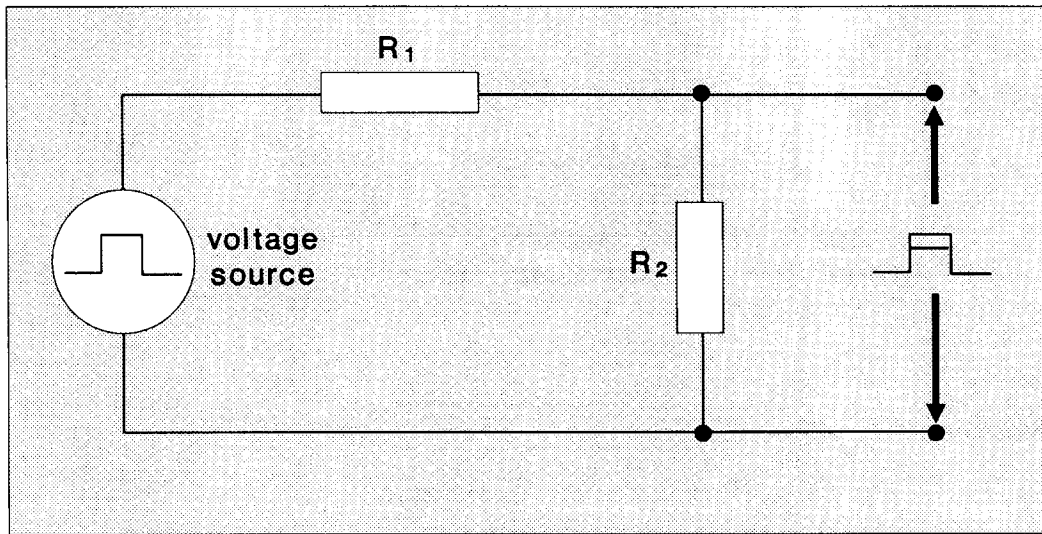


Fig. 3: Electric circuit with resistors only. Reduction of the amplitude; no changes in the impulse form

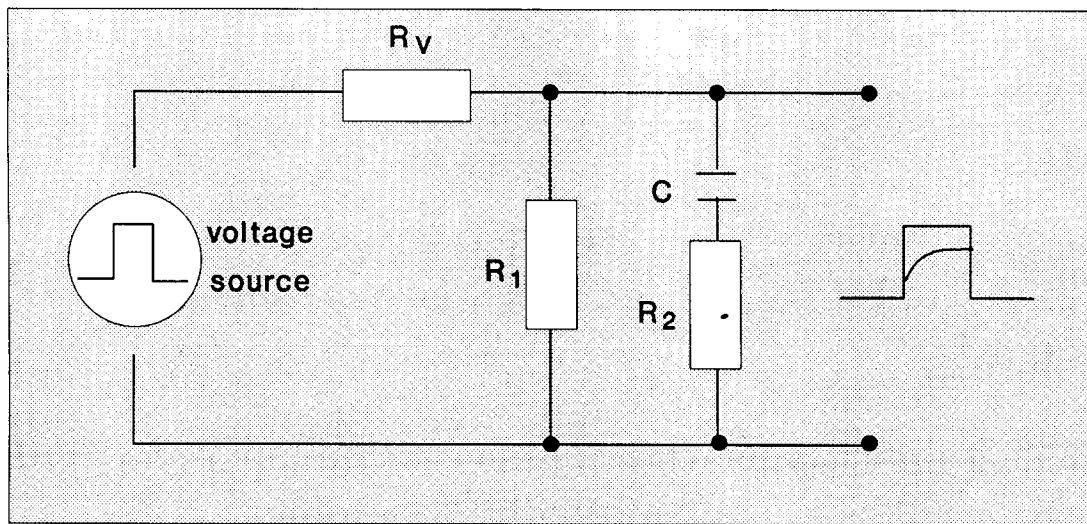


Fig. 4: Electric circuit with resistances and one condenser. Reduction of the amplitude plus changes in the impulse form

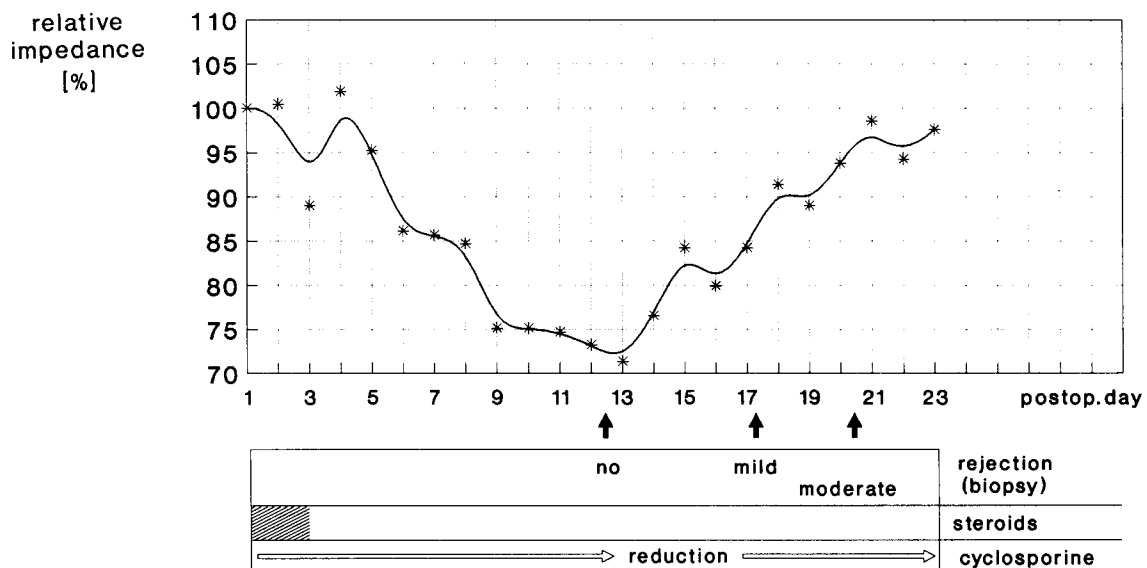


Fig. 5: Relative impedance after heterotopic heart transplant without treatment of rejection

relative impedance [%]

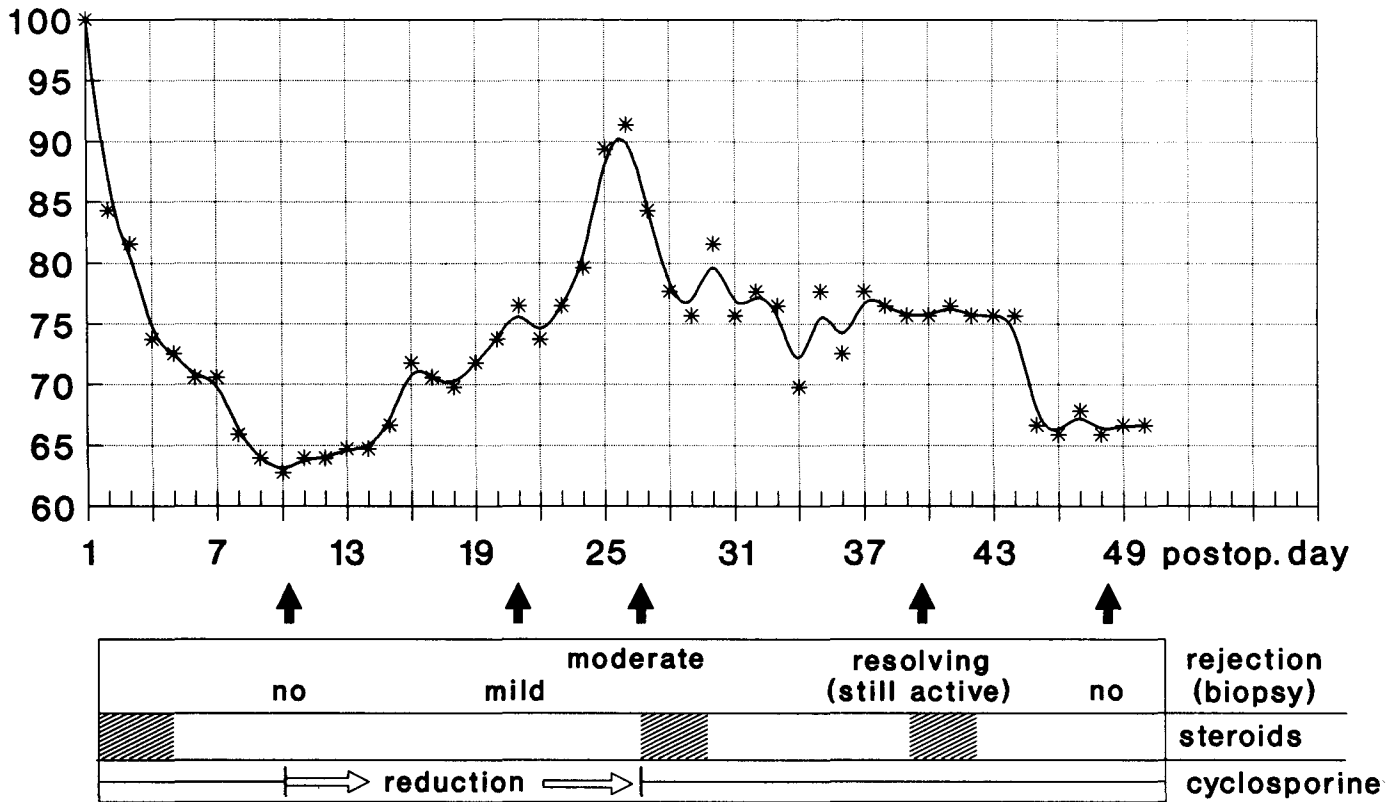


Fig. 6: Relative impedance after heterotopic heart transplant with treatment of rejection

constant phase of impulse

transient phase of impulse

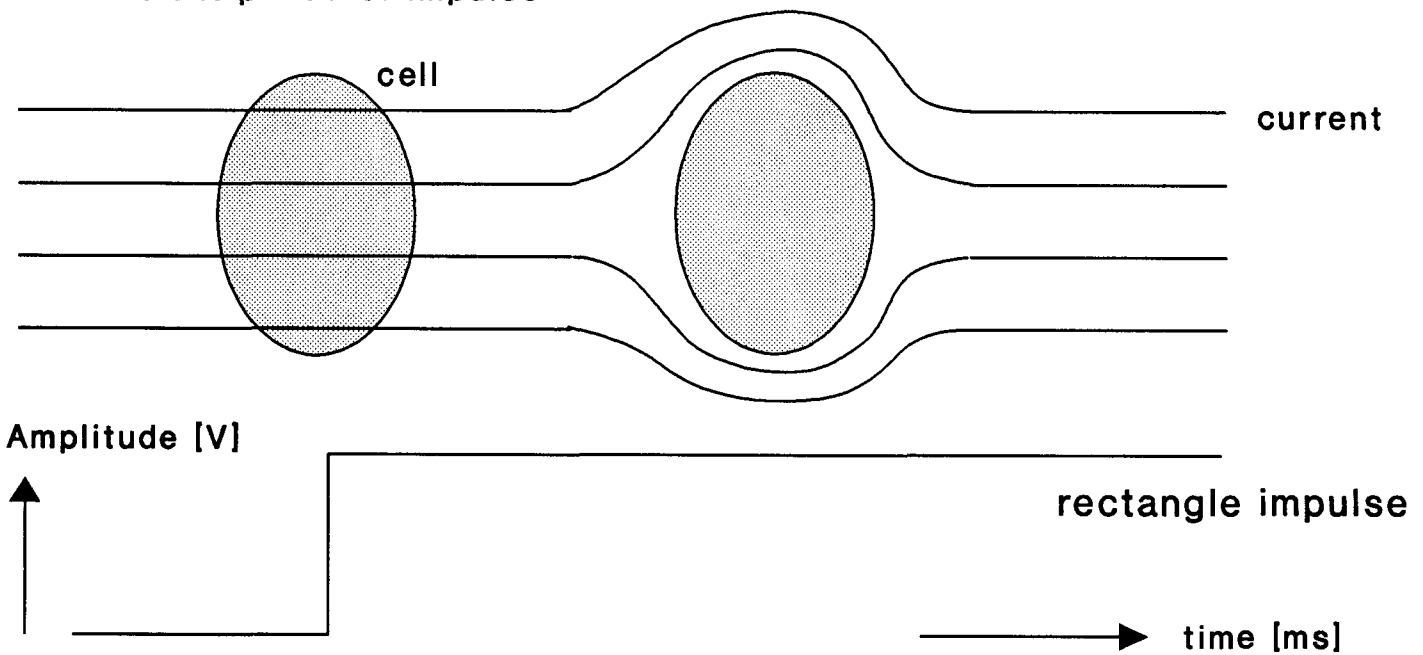


Fig. 7: Effect of an impulse on biological cells