



PC-Based Noninvasive Measurement of the Autonomic Nervous System

Detecting the Onset of Diabetic Autonomic Neuropathy

Diabetes is well recognized as a major health problem: a chronic disease caused by a lack of insulin and/or its effectiveness. Consequence of this disease are high blood glucose levels and other metabolic abnormalities that lead to short- and long-term complications. Diabetes mellitus afflicts more than 100 million people worldwide, and chronic diabetes complications are probably responsible for approximately one-third of all cases of blindness, renal disease, and lower limb amputation [1, 2]. Diabetic autonomic neuropathy may manifest as dysfunction of several different organ systems, e.g., cardiovascular, gastrointestinal, genitourinary, sudomotor and ocular.

Medical technology used to predict diabetic autonomic neuropathy is becoming increasingly more expensive, particularly for many developing and newly industrialized nations. To provide an inexpensive way for measurement of peripheral nerve function and vascular assessment, an IBM PC-based system based on noninvasive testing of transient autonomic responses has been developed. The system uses specialized hardware and software for neurophysiological measurement of auto-

nomnic dysfunction. Transient behavior of the autonomic response is measured to evaluate skin sudomotor and vasomotor sympathetic nerve dysfunction. The system also implements a fast and reproducible new method for measurement of the cardiovascular system and early detection of cardiac sympathetic and parasympathetic denervation.

The following quantitative noninvasive autonomic function tests have been validated and proved to be reliable and reproducible:

1. Tests of skin sympathetic sudomotor activity (SSA).
2. Tests of skin sympathetic vasomotor activity (SVA).
3. Tests of sympathetic/parasympathetic heart rate control (S/P HRC).

Results of these tests correlate with each other and with tests of peripheral somatic nerve function and have prognostic value. Thus, they provide a battery of fast quantitative measurements of autonomic function for simple and routine use in clinical practice and research.

IBM PC-Based Noninvasive Tests

The extensive studies that have examined the autonomic nervous (ANS) func-

Simion Pruna and A. Dumitrescu
Electrophysiology and Bioelectronics Group
Romanian Society for Clinical Engineering and
Medical Computing
"Dr. I. Cantacuzino" University Hospital, Romania

Table 1. Impedance reactometer specifications.

impedance range kohms	0 to 60
maximum nonlinearity, percent	1
output voltage range, V	10
resolution, ohms	1
output sensitivity, V/ohm	0.1
peak-to-peak noise, mV	10
setting times, sec	1.5
check of good electrode/skin contact	with individual indicating instruments for each channel measuring dermal impedance from 0 to 60 kohm
external acoustic stimuli	binaural with sound pulses of 860 Hz, 500 ms, 60 dB

tion, e.g., skin SSA, can be divided into two broad categories: (1) direct recording including microneurography, i.e., direct study of sympathetic activity from unmyelinated axons with intraneural recording needle electrodes, and (2) indirect recording based on attenuation of the physiological variables controlled by the ANS [3-5]. Microneurography is not applicable as a screening method because it is time consuming, requires skilled and experienced operators, and is invasive. In practice, any diagnostic test should be, first of all, simple and noninvasive.

Because the amplitude of the electrodermal response may be small, the measurement of skin response using DC excitation is not practical because of drift. A new technique for autonomic response assessment using a dual-channel self-balancing impedance reactometer has been developed in our laboratory [6, 7]. This system, based on lock-in detection and a self-balancing technique, responds to small changes of electrodermal parameters induced by activation of eccrine sweat glands by muscarinic cholinergic agents. Our electrodermal impedance measurement is based on AC excitation: a sine wave source with 860 Hz phase excitation, a self-balancing circuit controlled by negative feedback loops consisting of voltage-controlled attenuators, summing amplifiers, synchronous detectors, and integrators [6].

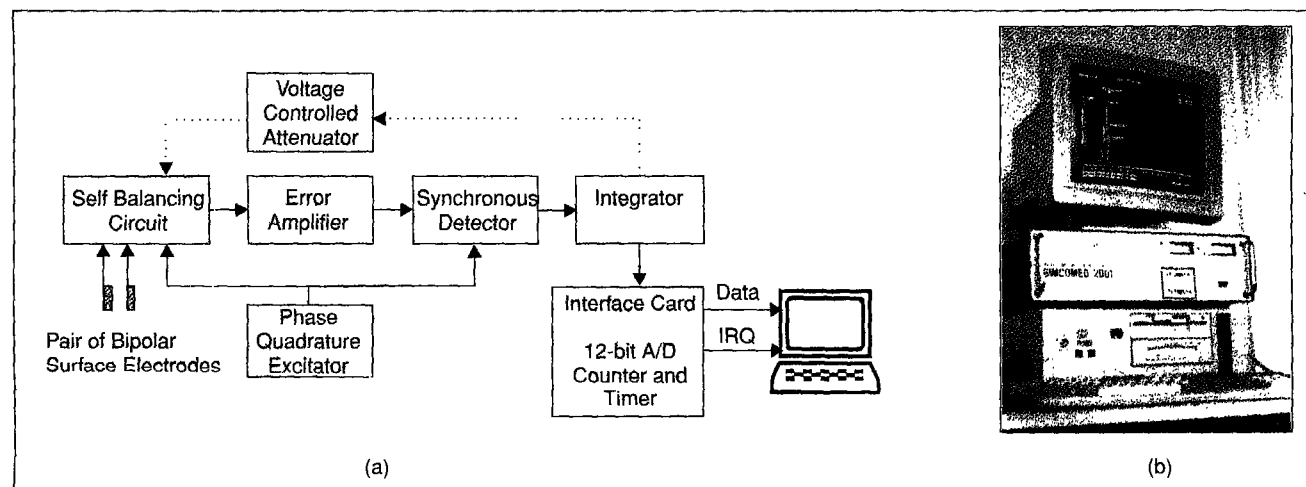
The physiological measurement principle of the impedance reactometer (Fig. 1) is based on the unbalance of the self-balancing circuit caused by the impedance change induced by the local skin SSA. The technique is automated by using

a feedback loop to provide in-phase and quadrature voltages for balance conditions. This automation allows continuous measurements of SSA and is essential, for example, in profile measurements of time-dependent drug effects (e.g., atropine) or autonomic behavior studies. Skin impedance fluctuations around equilibrium reflect the dynamics of SSA functional state under resting conditions or during stimulus-induced responses. The AC current, inversely proportional to local skin impedance, is converted to an AC voltage, which is then synchronously demodulated by the lock-in amplifier. The output is then filtered and the resulting phase-sensitive DC voltage provides an analog signal proportional to the SSA. Data acquisition is performed by relatively simple interface hardware, a PC 27 (Amplicon, Liveline Ltd.) low-cost, 16-channel, 12-bit I/O board consisting of a pulse counter, timer, and analog/digital converter (ADC). The interface program responds to the programmable timer interrupt request signals by reading the A/D converters and generating time-variable skin sympathetic sudomotor signals.

New software and automated analysis programs were developed for the clinical research. These programs, which run under DOS, allow us to compare data quantitatively and to aid in the differentiation of normal and abnormal SSA by time-domain analysis, peak detection, and successive local extreme amplitude display, which is a simple procedure intended to provide the clinical information. The sampling method is based on defining an interrupt that stores the sample data in memory and is triggered by the board at a

The sampling rate is independent of the hardware used, relying on the timers of the I/O board.

user-specified rate. The sampling rate is independent of the hardware used, relying on the timers of the I/O board. The acquisition board, together with a classic EGA display controller, allows sampling rates with values greater than 25 ms/cm. However, with a better graphic controller, the performance could be improved. The sampling is done in real-time so that the user can see the spot on the screen as the sampling progresses. The engineering menu allows the setting of various parameters of the program. It enables the setting of the time base and gains and channel calibration and allows channel selection. The gain option allows the channels to be individually controlled. Control of the analyzing sequence is provided through a separate set of buttons and available "help." The software functions allow the display of recorded traces, delete displays, and stepping back and forth.



1. (a) Block diagram of the IBM PC-based Impedance Reactometer; (b) photo of the system.

Direct analysis of the displayed trace is supported, and a specific function enables a cursor and its forward/backward step movement.

Help is available throughout the program and may be activated at any time when "F1 HELP" is highlighted at the bottom of the screen by pressing the F1 key. The system provides sensitive help that is relevant to the actual state of the program. If the page provided is not sufficient, further help may be called up by selecting another page. The program gives a description of the function of each procedure listed in the menu. The procedures are written to be as self-explanatory as possible, prompting the user for the exact information required, and rejecting whenever possible unsuitable or nonvalid entries by the operator.

System operation is conceived to have four phases. In phase 1 signals are digitized and stored on disk. Phase 2 represents data collection that is independent of the data analysis, so data remain unaffected by possible fatal errors occurring in the more sensitive analysis phase. Phase 2 reads the collected data, processes them, and provides results that are displayed and stored. In phase 3, using commercially available software packages, further analysis may be performed on the data-output of phase 2. Phase 4 generates a report, first as a screen display and then by printer. The clinical test database consists of approximately 20 items: basic patient information, name of disease, date of test, name of consultation department, results of autonomic tests, results of laboratory analysis, etc. In our system, there are diagnostic functions: normal, borderline, and abnormal, based on the normal distribution of laboratory data (mean \pm SD). Table 1 lists the technical parameters of the instrument developed.

Experimental Data

Some representative experimental results illustrate applicability of our approach to investigation of skin SSA, skin SVA, and S/P HRC.

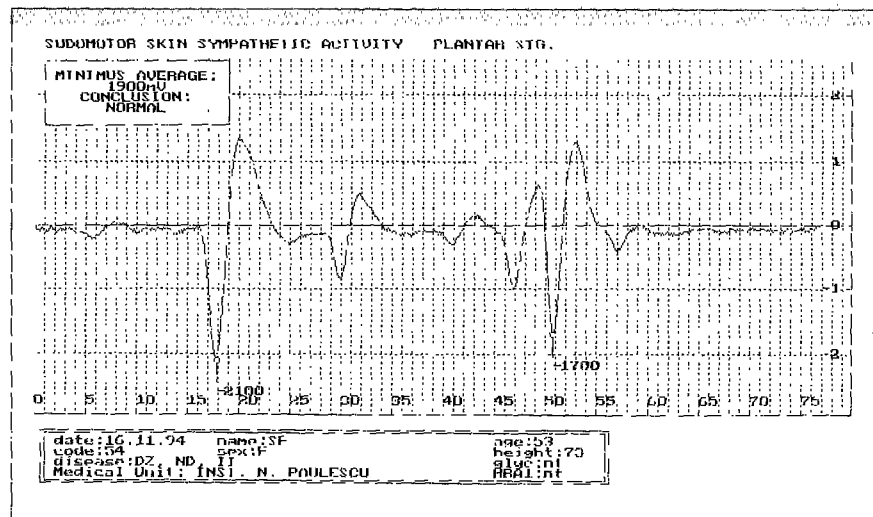
Tests of Skin SSA

Two surface cutaneous electrodes, connected to a self-balancing skin impedance circuit, were used to measure transient changes of skin impedance, as described above.

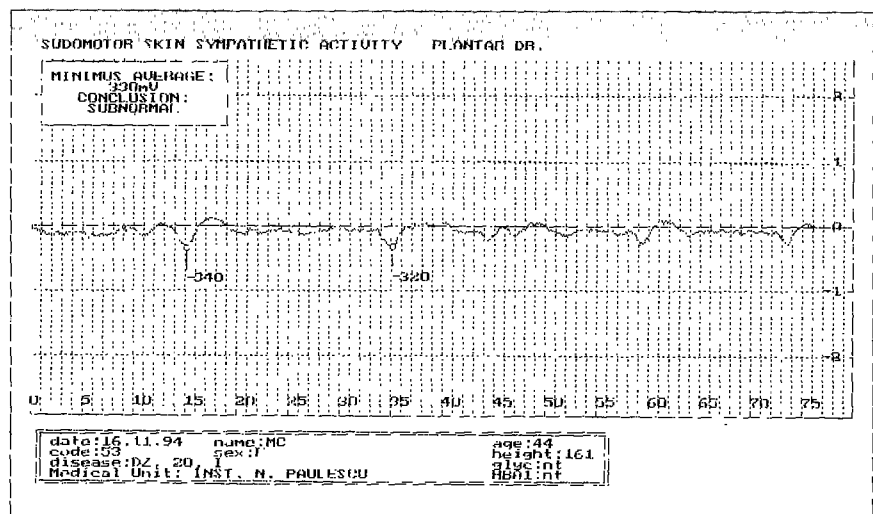
Measurements were made using the method described previously [8]. All measurements were performed in a simi-

lar way, with the subject laying in a bed in a relative acoustically isolated room. The emphasis here is dealing with illustrative material. Figure 2 shows consecutive SSA responses detected over the sole of the foot with the speed of recording (the time base) at 3 sec/cm. The subject was stimu-

lated by a 500 ms sound pulses of 860 Hz, 60 dB tone given to both ears. Well-defined response spikes can be seen, and the evoked responses are quite large with a high signal-to-noise ratio. Figure 3 shows an example of SSA responses in a patient presenting signs of autonomic neuropath-



2. Consecutive SSA responses detected over the sole at 3 sec/cm recording speed. Well-defined response spikes with large signal-to-noise ratios are seen.



3. SSA responses of a patient presenting signs of autonomic neuropathy, assessed as the absence of bradycardia on inspiration and the presence of orthostatic hypotension.

Table 2. Mean value and standard deviation of measured parameters.

	Left big toe	Right big toe	Left index finger
PPG (mV)	298.7 \pm 232.2	517.71 \pm 336.9	2217.1 \pm 1206.7
LDF (PU)	34.3 \pm 19.8	53.9 \pm 41.2	246.9 \pm 207.4
r	0.64	0.79	0.45
p	<0.002	<0.001	<0.001

thy, assessed as the absence of bradycardia on inspiration and the presence of orthostatic hypotension. It is clear that the two type of responses depicted in Figs. 2 and 3 are similar, but the latter has much lower amplitude according to the functional state of the ANS due to the diabetic neuropathy. The primary diagnostic information for autonomic neuropathy assessment corresponds to different SSA signal peaks. The SSA is tested by repeated application of the stimulus, and the assessment of the functional state of the

SSA is carried out by averaging at least the five most significant peaks. Usually, we use irregular time-interval delivery auditory stimuli to avoid subject habituation with stimulus. It is important to note that the traces obtained can be monitored continuously and seem to reflect SSA changes instantaneously, indirectly, and noninvasively. The measurements can be performed in a shorter time, with sensitivity and accuracy comparable to those made with the microneurography technique. The use of IBM PC-based signal

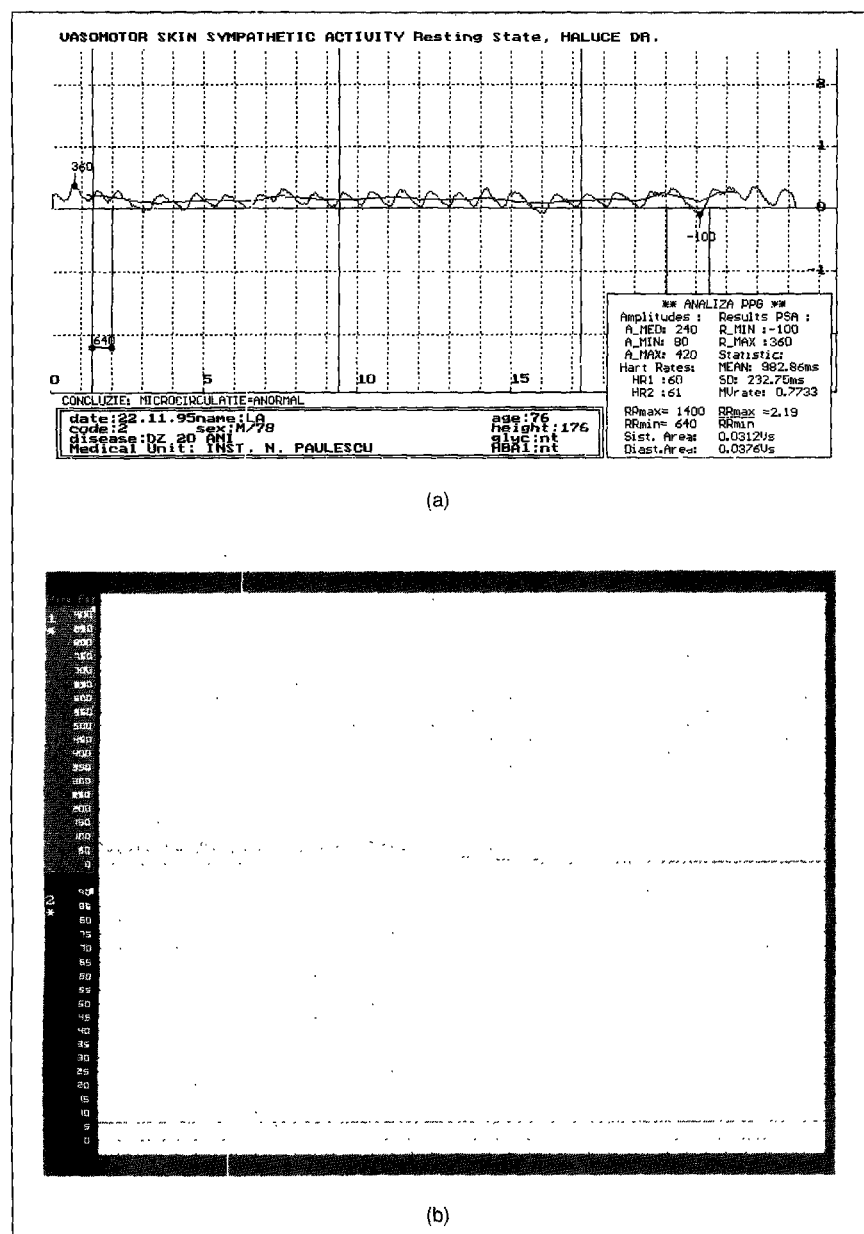
analysis makes the diagnosis process easier and faster.

Tests of Skin SVA

Apart from the skin SSA measurement, our system supports a variety of noninvasive procedures for the detection of in-vivo changes in sympathetic cutaneous vasomotor activity. An input connector is available for a photoplethysmographic (PPG) plug-in probe (visible, red, or infrared signal) to detect real-time skin transient pulse blood (STPB) signals and heart rate. The measurement technique discussed above points out that a self-balancing impedance (resistance for PPG detection) method automates the measurement of biosignals. Software for automated analysis of successive epochs of stored signals was developed for quantization of the measurements, with the specific aim of evaluating diabetic microangiopathy. Diabetic microangiopathy refers to the deterioration of capillaries and the control mechanism that regulates tissue perfusion. Changes in capillary blood flow are recorded in the basal state and during peripheral sympathetic adrenergic activation. These changes are dominant in small arteries and large arterioles, induced by both the transient vasodilator and vasoconstrictor reactions. Hardcopy of any graphical and numerical results may be recorded on a printer. The user can select an amplitude (time) or spectral (frequency) analysis.

To correlate the measurements both with clinical data and with classical measurements used to investigate diabetic microangiopathy, and to qualify our system's ability for early detection of skin blood perfusion impairment, our infrared PPG and a commercial laser Doppler flowmeter (LDF) were evaluated in a comparative study [9]. The LDF had 0.8 mW emission at 780 nm (Periflux System 4001, Perimed, Sweden). Our infrared PPG probe assembly uses a spectrally matched near-infrared emitter and sensor. The emitter has 15 mw output at a wavelength of 950 nm, and the sensor is a high-speed, highly sensitive PIN photodiode, housed in a black infrared transmissive molding that reduces ambient white light interference.

All measurements were performed with the patient in the supine position, resting comfortably on an examination bed, with a mean room temperature of 25°C. The probe was fixed to the skin of the big toe by means of double-sided ad-



4. (a) An example of the screen display of STPB recording in measuring the peripheral microcirculation using the IBM PC-based system. The signal (right big toe) was recorded from a patient with diabetic microangiopathy. (b) Screen display for LDF in measuring peripheral microcirculation using the Perimed system. The signal was recorded from the same patient (right big toe).

hesive, and the probe lead was secured by surgical tape to prevent movement artifact. Changes in capillary blood flow were recorded in the basal state and during peripheral sympathetic adrenergic activation dominant in small arteries and large arterioles, induced by both the transient vasodilator and vasoconstrictor reactions.

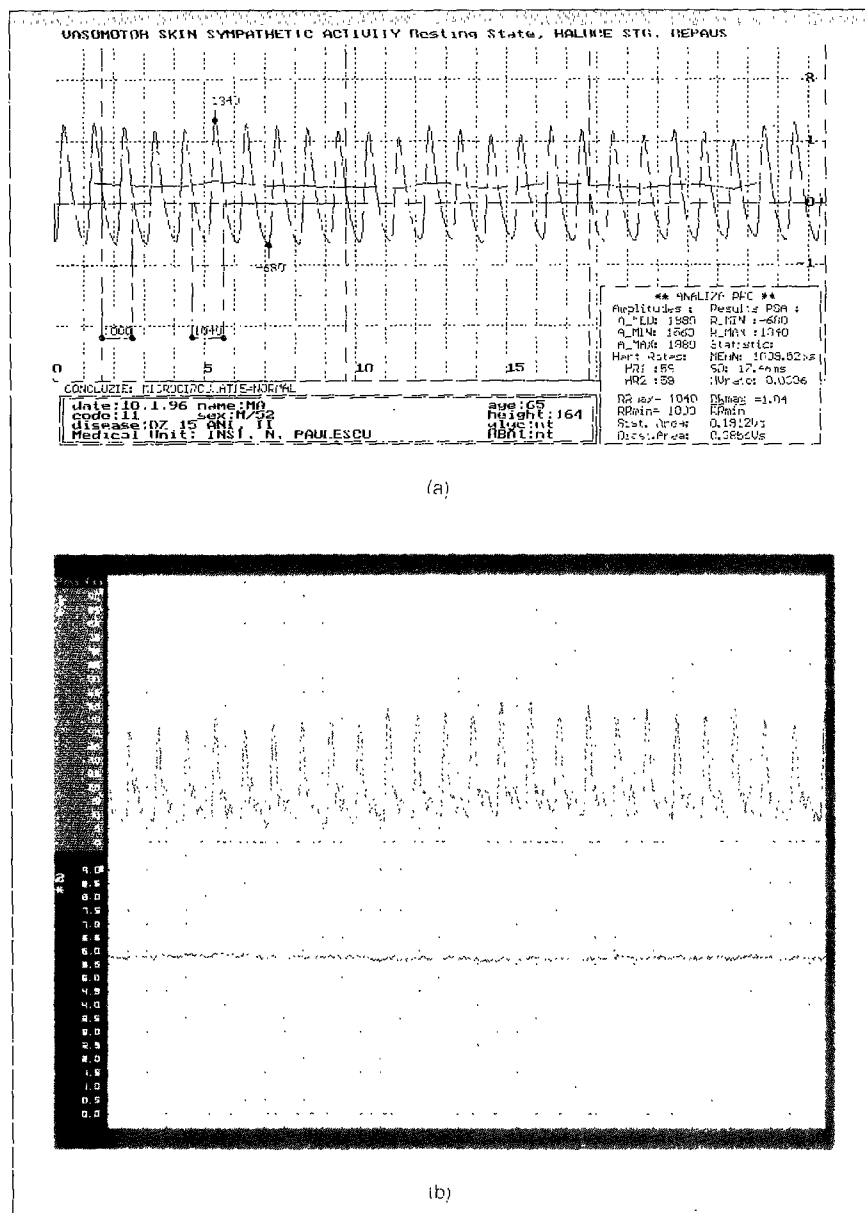
Automated software analysis was developed for the quantization and measurement of successive epochs of stored signals. Skin blood perfusion was measured in 29 Insulin-dependent diabetic mellitus selected patients (mean age 37.65 +/- 14.7 yrs., duration of diabetes 15.76 +/- 8.38 yrs.). Measurements were made successively at the same anatomical sites to measure blood flow in the same vascular bed: left big toe, right big toe, and left index finger, all at the same local skin temperature. Two sets of measurements: average PPG amplitude (mV) and LDF perfusion units (PU) were made with each subject, each measurement lasting 26 sec, i.e., about 35 HR cycles.

Figure 4 shows the STPB and LDF recorded on a male subject (left big toe) with diabetic microangiopathy. Figure 5 shows an example of the screen display of STPB and LDF, recorded from a male (right big toe) without diabetic microangiopathy. There was a significant correlation between the average PPG amplitude, expressed in mV and LDF, expressed in arbitrary PUs, at each anatomical site, as is shown in Table 2. Comparison of results of measurements obtained with STPB and LDF are presented as a 2D histogram in Fig. 6.

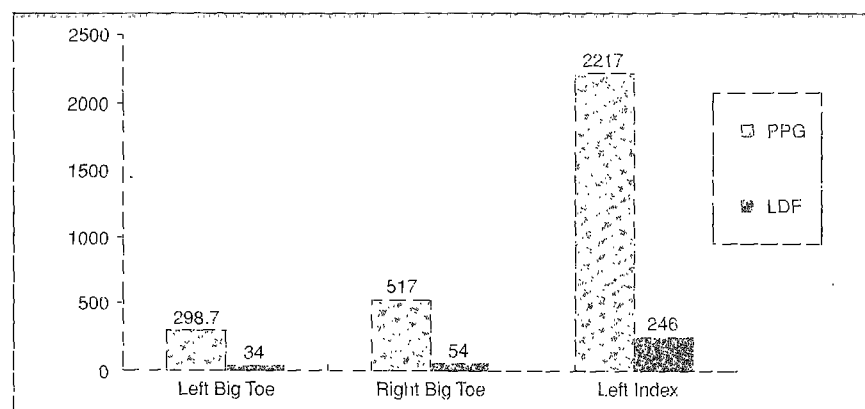
These results have indicated that the PPG and LDF are equally suitable for use in the diagnosis of skin blood perfusion impairment at foot level in diabetes. However, the PPG system is far less expensive than the LDF.

Sympathetic/Parasympathetic Heart Rate Control Tests

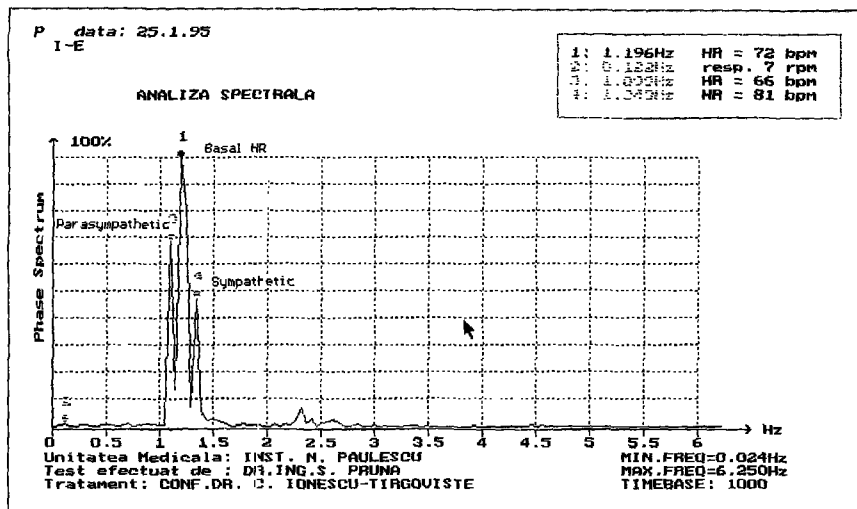
Because of the dual innervation of the heart, it is important to investigate the possibility of distinguishing between sympathetic and parasympathetic diabetic impairment by applying noninvasive quantitative measurements. As heart rate oscillates in synchrony with respiration, several methods have been employed to assess this "sinus arrhythmia" as an index of autonomic nervous system function. Although R-R variation measurement is one of the most commonly used techniques in diagnosing diabetic autonomic



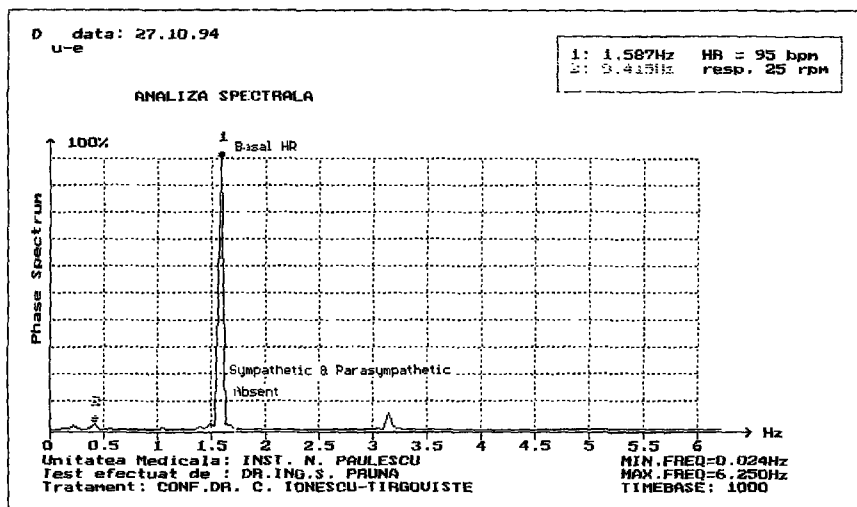
5. (a) An example of screen display for STPB recording in measuring the peripheral microcirculation using the IBM PC-based system on a patient without diabetic microangiopathy. (b) Screen display for LDF in measuring peripheral microcirculation using the Perimed system on the same patient.



6. Comparison of results of measurements obtained with STPB and LDF.



7. FFT spectrogram of STPB recording during controlled inspiration-expiration from a patient without autonomic neuropathy.



8. FFT spectrogram of STPB recording during controlled inspiration-expiration from a patient with autonomic neuropathy.

neuropathy [10-12] because it shows good reproducibility and high sensitivity [13,14], it is difficult to distinguish between the sympathetic and parasympathetic influences [14]. Drugs such as propranolol, which blocks β -adrenoceptors, may be used to study sympathetic influence on heart rate, but quantitative assessment is difficult. More direct methods of studying sympathetic activity record impulses in nerves by microneurography or by measuring plasma noradrenaline levels. Microneurography is not applicable as a routine because it is time consuming, requires skilled and experienced operators, and is invasive. Tests relying on plasma noradrenaline responses in clinical practice may be disabling and difficult, and thus are usually performed on animals. This

limitation has led us to develop a noninvasive test for routine screening in the investigation of autonomic dysfunction: time (rate of change) and frequency (spectral) analysis of transient phenomena in autonomic behavior. Recently, we introduced a more advanced method that enables separate assessment of parasympathetic and sympathetic activities by using spectral analysis. Our equipment, hardware and software, was tested in institutions in Bucharest and other cities in Romania.

In the frequency range 0.5-2 Hz, the technique was able to detect a frequency-modulated heart rate, typically characterized by two spectral sidebands, during controlled inspiration in healthy subjects and diabetics without autonomic neuropathy; and a single or no spectral

sideband in patients with early and severe autonomic neuropathy, respectively. Since the STPB amplitude may vary by orders of magnitude, the peak amplitude corresponding to basal heart rate (f_0) was normalized as 100%, and the relative heart rate variability (RHRV) was defined as $(f-f_0)/f_0 \cdot 100\%$. A frequency-modulated heart rate was detected, characterized by two spectral sidebands defined as sympathetic (f_s) and parasympathetic (f_p) frequencies. We use the maximum/minimum ratio, defined as the longest R-R interval divided by the shortest interval. Early detection of cardiac autonomic dysfunction was done using the RHRV, as measured by the fast Fourier transform (FFT) of the transient (first derivative) AC component of the STPB (near infrared) signal contained in the frequency range 0.5-2 Hz. Figure 7 shows the typical FFT spectrogram of STPB recordings during controlled inspiration-expiration from a patient without cardiac autonomic neuropathy. It can be seen that under controlled respiration, the patient undergoes two frequency shifts, which we suggested to be the effect of sympathetic (right shift) and parasympathetic effects (left shift) associated with sinus arrhythmia. Our observation and results indicate that as the severity of autonomic dysfunction increases, the frequency shifts decrease because of the autonomic cardiac neuropathy. In addition, the peak power spectrum decreases proportional to sympathetic/parasympathetic dysfunction, as illustrated in Fig. 8.

Conclusions

The IBM PC-based system and experimental results presented here describe a new approach to recording electrophysiological signals associated with the autonomic nervous system, and the detection of the changes in electrophysiological signals due to onset of diabetic autonomic neuropathy. We have shown that the SSA has much lower amplitude due to the functional state of the ANS caused by the diabetic neuropathy. The STPB amplitude over many sweeps is statistically correlated with the LDF amplitude, but the PPG technique is less expensive. Spectral analysis of STPB in the frequency range 0.5-2 Hz enables the separate assessment of parasympathetic and sympathetic activities and the rapid detection of cardiac autonomic neuropathy.

Acknowledgment

The authors are grateful to the Commission of the European Communities for financial support through the auspices of the BIOMED and PECO93 programs for 1993-1995. Special thanks are due to Dr. V. Thevenin, the CEC contact person for research on biomedical technology and engineering.



Simion S. Pruna carries out multidisciplinary research in the application of electrophysiological measurement, computer programming, electronic equipment design, and data base management systems to electrophysiological measurement of diabetes complications in the Electrophysiology and Bioelectronics Laboratory, Institute of Diabetes "N. Paulescu" in Bucharest. He received the M.S. in Electronic Engineering in 1971 and the Ph.D. degree in 1992 from the Politechnical University, Bucharest. He has developed hardware and software for the multiparameter investigation and assessment of the autonomic nervous system and the cardiovascular system. The main field of measurement techniques development is in the in vivo noninvasive investigation and assessment of the autonomic nervous system and cardiovascular system, an area in which Dr. Pruna continues to focus his research activity. Dr. Pruna is a member of the European Society for Engineering and Medicine (ESEM), International Association for Study of Pain (IASP), European Group for Study of Diabetes, European Association for the Study of Diabetes (EASD), IMEKO Technical Committee TC 13, and a Founder Member of the European Association for the Study of Acute Abdominal Pain.



Andreea Dumitrescu received her engineering education B.E. in 1990 and M.Sc. in 1992 from the Politechnical University, Informatics and Automation, in Bucharest, Romania. Mrs. Dumitrescu then worked for the Electrophysiology and Bioelectronics group of The Romanian Society for Clinical Engineering & Medical Computing, involved in the development of a software package for the IBM PC, which combines the functions of a storage oscilloscope, voltmeter, and spectrum analyzer for signal acquisition and analysis and provides a data base for both patient testing and monitoring the effects of treatment. Andreea is member of Romanian Society for Clinical Engineering and the Romanian General Association of Engineers.

Address for Correspondence: Simion Pruna, Electrophysiology and Bioelectronics Group of the Romanian Society for Clinical Engineering and Medical Computing, "Dr. I. Cantacuzino" University Hospital, Movila, 5-7, 79811, Bucharest, Romania.

References

1. Diabetes Control and Complications. Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes. *New Eng J Med* 329:977-986, 1993.
2. Consensus Statement: Report and recommendations of the San Antonio Conference on diabetic neuropathy. *Diabetes*, 1988; 37: 1000-1004.
3. **Fagius J:** Microneurographic findings in diabetic polyneuropathy with special reference to sympathetic nerve activity. *Diabetologia*, 1982; 23: 415-420.
4. **Berne C and Fagius J:** Skin nerve sympathetic activity during insulin induced hypoglycemia. *Diabetologia*, 29, 855 - 860.
5. **Calancie BM, Stein RB:** Microneurography for the recording and selective stimulation of afferents: an assessment. *Muscle and Nerve*, 1988; 11: 638-644.
6. **Pruna S, Ionescu-Tirgoviste C:** Dual-channel self-balancing electrodermal impedance reactometer for autonomic response studies. *Med & Biol Eng & Comput* (1987) 25, 613-619.
7. **Pruna S:** Improved Technique for Testing Autonomic Dysfunction: Evaluation of Transient Behaviour of the Autonomic Response. *Med & Biol Eng & Comput* 1990, 28 119-126.
8. **Ionescu-Tirgoviste C, Pruna S:** Quantitative noninvasive electrophysiological evaluation of the activity of the cutaneous division of the sympathetic nervous system. *Arch Int De Physiologie Et De Biochimie*, 1990, 98, 111-119.
9. **Pruna S, Ionescu-Tirgoviste C, and Mincu I:** A comparative study between a new PC-based infrared photoplethysmography and a laser Doppler flowmetry (Abs.). 3rd Annual Meeting, European Association for the Study of Diabetes, Vienna, Austria. *Diabetologia* Vol 39, Suppl. 1, August 1996, pp A216.
10. **Weinberg CR and Pfeifer MA:** Development of a predictive model for symptomatic neuropathy in diabetes. *Diabetes* (1986), Vol 35, No 8, 873-880.
11. **Ewing DJ, Martin CN, Young RJ and Clarke BF:** The Value of Cardiovascular Autonomic Function Tests: 10 Years Experience in Diabetes. *Diabetes Care*, (1985) Vol 8, No 5: 491-498.
12. **Schumer M, Miller-Crain G, Pfeifer MA:** The Statil Study Group (1988) diabetic autonomic neuropathy - Part II. Coefficient of variation of RR-variation and Valsalva manoeuvre tests. *Am J Med* 85 (Suppl 5A) : 144-146.
13. **Ewing DJ, Clarke BF:** Diabetic autonomic neuropathy: A clinical viewpoint. (in) Dyck PJ, Thomas PK, Asbury AK, Winegrad AI, Porte D (eds) *Diabetic Neuropathy*, WB Saunders, Philadelphia, 1987, pp 66-88.
14. **Genoveli H and Pfeifer MA:** - RR-variation: autonomic test of choice in diabetes. *Diabetes/Metabolism Rev.*, (1988), 4, (3), 255 - 271.